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Original communication

Comparison between prostate specific antigen and acid phosphatase for detection of semen in vaginal swabs from raped women

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ABSTRACT

Objective: To compare the effectiveness of the prostate specific antigen (PSA) test and the acid phosphatase (AP) test for semen detection in human vaginal samples.
Material and method: The source materials were vaginal swabs that were tested at Ramathibodi Hospital between 2008 and 2010 from 2450 cases of raped women. Each swab was tested for semen by three methods: sperm detection by light microscopy, the AP enzymatic reaction, and the presence of PSA by using an immuno-chromatographic rapid kit test. The efficiencies of the AP and PSA tests were compared using the light microscopy result for the presence of sperm as the gold standard.
Result: The specificities of the AP, the PSA and the combined AP-PSA tests were 96.4%, 92.3% and 91.9%, respectively, and the sensitivities were 65.5%, 80.4% and 84.5%, respectively. The receiver operating characteristic (ROC) area of the AP, PSA and combined AP-PSA tests were 0.8091, 0.8639 and 0.8823, respectively. The ROC area of the PSA test was significantly greater than that of the AP test ($p < 0.0001$), and the ROC area of the combined AP-PSA test was significantly greater than both the tests individually ($p < 0.0001$).
Discussion: Based on the ROC area, the PSA test was better than the AP test for semen detection in the vaginal swabs, and the combined results (AP + PSA) were better than the individual tests. The specificity of the AP test was higher than the PSA test in this study because a positive detection was made within only 15 s. While the PSA test was more convenient as it was available in a rapid test kit format, our recommendation is PSA detection should be done together with AP test and spermatozoa examination to identify evidence of rape.
Conclusion: Using these three tests together (AP, PSA, and spermatozoa detection) was recommended as a forensic tool for investigations of vaginal swabs of the rape victims.

1. Introduction

Sexual assault crimes involve physical contact between the perpetrator and the victim and consequently exchange of biological material such as hair and body fluids, particularly seminal fluid. Proven evidence of the presence of these biological materials can identify the perpetrator and assist the judge in making a decision.

Detection of the presence of spermatozoa examination and acid phosphatase (AP) has been widely used in forensic analysis of rape investigation in many countries.[1] AP activity, is a sensitive and specific screening or presumptive test, as it may be detected in the vagina up to 72 h post-coitus[2] whilst examination for presence of spermatozoa is highly specific as sperm may be detected in the vagina for up to 7 days after intercourse, so this is often used as a confirmatory or 'gold standard' test.[3] However, AP occurs naturally in the vagina at low concentration[4] and this would cause a false positive result.

The prostate specific antigen (PSA) test was introduced into rape investigation in 1971.[5,6] Although female bodily secretions such as breast milk, and sweat do contain PSA, the levels are usually below the limit of detection for this test which reduces the likelihood of false positive.[7]

PSA detection is regarded as a useful confirmatory test in rape investigations because it can persist at detectable levels in the vagina for up to 48 h postcoitus.[8] Many different commercial kits for PSA detection have been developed. The ELISA technique was

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introduced into PSA detection in 1991.[9] One of the most widely used techniques is a rapid one-step immunochromatographic assay because of its convenient and the accuracy and specificity were close to the ELISA technique.[10–12] However, there were some reports that demonstrate the false positive results in a few cases.[13,14]

This study compared AP activity (by the brentamine fast blue test)[15–17] and presence of PSA (detection by a rapid one-step immuno-chromatographic assay)[11,12,18–21] with light microscopic examination for the presence of spermatozoa, which is regarded as the 'gold standard' to assist in rape investigations.

2. Materials and methods

The laboratory examination results of 2450 vaginal swabs from raped women which were sent to Ramathibodi hospital during 2008–2010 were studied retrospectively. The swabs were taken by hospital physicians, so we did not know the real situation except only history of vaginal penetration. One vagina swab from each case was tested by 3 methods: acid phosphatase (AP) activity, prostate specific antigen (PSA) detection and spermatozoa examination.

2.1. Acid phosphatase (AP) activity

AP was detected by the brentamine fast blue test. A small piece of cotton was cut from vagina swab and was put in the well of the AP test tray. One part of solution A and nine parts of solution B were mixed and filtered (daily prepared). The mixed solution was dropped onto the cotton. Positive appearance of purple color within 15 s indicated AP activity. A positive control (known semen stain) and a negative control (distilled water) were included in each test batch to ensure that the reagents were working properly.

2.2. Prostate specific antigen (PSA) detection

PSA was detected by immunochromatographic assay, a rapid one-step semi-quantitative method. The PSA commercial kit was from Advanced Quality (Code ITP 07002-TC40), InTec PRODUCTS, INC (XIAMEN), P.R.China. The PSA test card is a nitrocellulose membrane treated with mouse anti-human PSA monoclonal antibody in the test region, control PSA in the control region, and antibody-colloidal gold conjugate in the sample well region. The remaining cotton from vagina swab was soaked in 2 ml buffered saline and they were mixed in the centrifuged tube for 10 min. Then, 100 μl of this solution was poured into the sample well of the PSA test card. The sample and the conjugate migrate onto the chromatographic membrane by the capillary action. If PSA is presence in the sample, a pink/purple band of antibody-PSA-colloidal gold complex will appear within 10 min in the test region and another pink/purple band of antibody-control PSA-colloidal gold complex will appear in the control region. If PSA is not present in the sample, the pink/purple band will only occur in the control region. The assay is optimized to detect 4 ng/ml of PSA. The test results were interpreted as follows:

Positive: Color bands occur in the test and the control regions.
If the intensity of color in test and control bands are equal, then [PSA] = 4 ng/ml;
If the color of the test band is stronger than the control band then [PSA] > 4 ng/ml;
If the test color is weaker than the control then [PSA] < 4 ng/ml.
Negative: Only one color band occurs and is in the control region.
Invalid: No color band occurs in the control region.

2.3. Spermatozoa examination

Spermatozoa examination was performed by light microscopy. Two milliliters of 25% NH_3OH solution were added into the remaining solution from PSA test and they were mixed for 10 s. The tube was left at room temperature in a fume hood for approximately 8 h and mixed again for 10 s. The cotton bud swab was removed by applicator stick. Spermatozoa were concentrated by centrifugation at 2500 rpm for 10 min. The supernatant was discarded, and approximately 25 μl of liquid was retained to resuspend the sediment. The sediment was smeared onto microscopic slide, dried and treated with papanicolaou stain (Papanicolaou's solution 1a − Harris' hematoxylin solution + Papanicolaou's solution 2a − Orange G solution (OG6) + Papanicolaou's solution 3b polychromatic solution (EA50), Merck) and examined at 400× magnifications. The presence or absence of spermatozoa was recorded.

2.4. Data analysis

The specificity, sensitivity, receiver operating characteristic (ROC) area, positive predictive value (PPV) and negative predictive value (NPV) of AP, PSA and combined AP-PSA techniques were analyzed by STATA statistics/Data analysis special edition 11.1. (Copyright 2009 StataCorp LP, StataCorp, USA) using spermatozoa examination as the gold standard. For the combined techniques, a positive result from one, two or all three techniques was considered to be a positive. The value of ROC closest to 1 was considered to be the best technique. The results of the analytical tests were compared using Chi-square test. A p value less than 0.05 was considered statistically significant.

3. Results

Table 1 showed the comparison of each technique for semen detection. It was found that spermatozoa examination and PSA test gave almost the same detectable rate in semen detection (24.8% by spermatozoa vs. 25.7% by PSA) and both of these two techniques were higher than AP test (19.0%). By using two techniques, combined AP-PSA test gave slightly higher detection rate (27.1%) than the spermatozoa and PSA tests.

The specificity, sensitivity, ROC, PPV and NPV of AP, PSA, and combined AP-PSA are shown in Table 2. The specificity and NPV of these three techniques were similar, i.e., the specificities were 96.4%, 92.3% and 91.9%, respectively. In contrast, the AP test was less sensitive than the PSA and combined AP-PSA tests, i.e., the sensitivity of AP, PSA and combined AP-PSA test were 65.5%, 80.4% and 84.5%, respectively.

The ROC area of the PSA test was significantly larger than AP ($p < 0.0001$) and the ROC area of the combined AP-PSA test was significantly higher than both AP and PSA test ($p < 0.0001$) (Fig. 1).

Table 1
The detection rates of spermatozoa, AP and PSA in vaginal swabs from rape victims ($n = 2450$).

Technique	No of positive	% of detection
Spermatozoa examination	608	(24.8%)
Seminal fluid detection		
Acid phosphatase (AP) test	466	(19.0%)
Prostate specific antigen (PSA) test	629	(25.7%)
Combined AP-PSA test[a]	663	(27.1%)

[a] A positive in at least one technique was considered as a positive sample.

Table 2
Specificity, sensitivity, ROC, PPV, and NPV of Acid Phosphatase (AP), Prostate Specific Antigen (PSA) and combined AP-PSA in tests of vaginal swabs from rape victims ($n = 2450$).

Technique	Specificity	Sensitivity	ROC area	PPV	NPV
Acid phosphatase (AP)	96.4%	65.5%	0.8091	85.6%	89.4%
Prostate specific antigen (PSA)	92.3%	80.4%	0.8639*	77.6%	93.5%
Combined AP-PSA	91.9%	84.5%	0.8823*	77.5%	94.7%

*$p < 0.0001$ compared with AP.

4. Discussion

This study is one of the large numbers of casework of raped women research (2450 cases). Although the detection of spermatozoa has been accepted as the gold standard for forensic evidence of semen, the test will be negative for vasectomized or oligospermic individuals. In addition, spermatozoa can easily deteriorate in an inappropriate environment. The AP and PSA methods are alternatives which offer the possibility to improve the effectiveness of semen detection.

AP and PSA are both substances that are produced by the prostate gland and then secreted into the seminal fluid. They usually co-occur with spermatozoa in semen ejaculated during coitus. However, the principles of detection by each technique are different. The AP test determines the activity of the AP enzyme by color change, whereas the PSA test detects the concentration of PSA by immuno-chromatographic assay.

The purple compound developed by the AP enzymatic reaction test is unstable. The technique requires experienced personnel, and delay in reading will cause a false positive result.[4] Reduction of interpretation time to 15 s from 60 s, that is used in many laboratories, diminishes the likelihood of a false positive[22] and increases the specificity, but it also decreases the sensitivity of the test.

In this study, the specificity of AP technique was 96.4%, which was similar to a previous report,[23] but the sensitivity was only 65.5%.

The one-step immuno-chromatographic assay for PSA detection is available as commercial kits, which have ability to eliminate false positive result due to low concentration of non-seminal PSA.[19,21] This technique is highly specific for seminal fluid and is an accepted confirmatory test for rape investigations.[9,19] The specificity of the PSA test in this study was 92.3%, that was similar to previous report.[24] However, in another study of consensual cases the specificity of PSA was as high as 97–99%.[25]

The results of other forensic test for sexual activity have to be compared with the spermatozoa test, as gold standard, in rape investigations. However individuals with vasectomy or oligospermia will cause difficulty in interpretation. The large sample size that was used in this study will minimize the error associated with these factors.

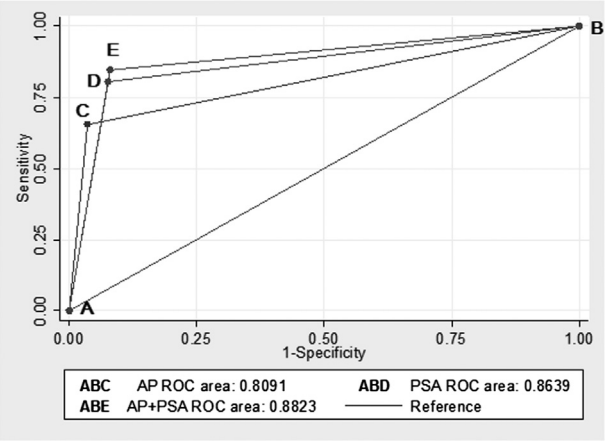

Fig. 1. ROC area of AP (0.8091), PSA (0.8639), and combined AP-PSA (0.8823).

This study showed that the ROC for the PSA technique was significantly greater than for AP. This finding is similar to some other studies.[9,26] Therefore, it referred that the PSA test is better than the AP test for semen detection. However, some guidelines for rape detection[27,28] and recommendation from some investigators suggested that using both AP and PSA will get the best result.[1,29] In the same way as this study, the ROC of the combined AP-PSA test was significantly higher than either the PSA test or the AP test with $p < 0.0001$ (Table 2, Fig. 1).

The detection rate of the PSA test was slightly less than the combined AP-PSA test (25.7% vs. 27.1%; Table 1), but greatly more than AP test alone (19.0%). Other advantage of the PSA test is the availability of rapid one-step commercial kits, which can be used to test small or large numbers of samples. Therefore, our recommendation is PSA detection should be done together with spermatozoa examination to identify evidence of rape.

However, the limitation of our study was lack of some useful information such as time passing from the rape occurring to the sample collection, the use of condom, intra- or extravaginal ejaculation, and number of perpetrator. Thus, we cannot provide the further discussion regarding relation of the tests (AP, PSA and spermatozoa) and the information above.

5. Conclusion

This study revealed that the PSA detection by immunochromatographic assay was better than the AP detection by enzymatic assay. However, the combine AP-PSA test was better than either the single AP test or the single PSA test. From this study, the PSA test had better sensitivity and negative predictive value, and the AP test had better specificity and positive predictive value. While the AP test had false positive result and complicated procedure, the PSA test had quite lower false positive result and was more convenient. Overall, using these three tests together (AP, PSA, and spermatozoa detection) was recommended as a forensic tool for investigations of vaginal swabs of the rape victims.

Ethical approval

This document has been approved by the Ethical Committee on Human Rights Related to Research Involving Human Subjects, Faculty of Medicine Ramathibodi Hospital, Mahidol University (MURA2011/569).

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Conflict of interest

The authors declared no conflict of interest.

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